α-ADRENERGIC STIMULATION OF [1-^{14}C]OLEATE OXIDATION TO $^{14}CO_2$ IN ISOLATED RAT HEPATOCYTES

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1. Introduction

Vasopressin and the catecholamines have a number of similar actions in rat liver. Both activate glycogenolysis [1,2–5] and phosphorylase [2–7,8] and stimulate gluconeogenesis [3,9–11]. Although the catecholamines increase the hepatic content of adenosine 3′,5′-cyclic monophosphate (cAMP) (the β-adrenergic response) the stimulation of glycogenolysis and gluconeogenesis in rat hepatocytes by adrenaline and noradrenaline is mediated mainly by α-receptors, possibly via a rise in the [Ca^{2+}] in the cytoplasm (reviewed [12]). Ca^{2+} has been implicated also in the actions of vasopressin on hepatic glycogenolysis and gluconeogenesis (reviewed [13]).

The site of action of the catecholamines and vasopressin on glycogen metabolism and gluconeogenesis is extramitochondrial. In addition, vasopressin has an intra-mitochondrial site of action, the increased oxidation of [1-^{14}C]oleate to $^{14}CO_2$ [14]. This action of vasopressin is Ca^{2+}-dependent. Here we show that the catecholamines adrenaline and noradrenaline also increase oxidation of [1-^{14}C]oleate to $^{14}CO_2$. The increased $^{14}CO_2$ production is blocked by the α-antagonist phentolamine, and is dependent on the presence of Ca^{2+} in the incubation medium.

2. Materials and methods

2.1. Reagents

All enzymes, coenzymes and substrates were obtained from BCL, Lewes, East Sussex. Adrenaline

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was from BDH, Poole, Dorset. Noradrenaline was from Winthrop, Surbiton-upon-Thames, Surrey. U. K. Phenylephrine was from Boots, Nottingham. Phentolamine mesylate BP (Rogitine) was from CIBA, Horsham, Sussex. Propranolol–HCl BP (Inderal) was from Imperial Chemical Industries, Macclesfield, Cheshire. Radiochemicals (including cAMP assay kits) were purchased from the Radiochemical Centre, Amersham, Bucks.

2.2. Preparation of hepatocytes

Female Wistar rats (180–250 g) were fed ad libitum on standard laboratory diet. They were anaesthetized with nembutal (60 mg/kg wt, solution in 0.9% NaCl). Isolated hepatocytes were prepared essentially as in [15] and modified as in [16]. Preparation of hepatocytes was commenced between 0.9:30 and 10:30 h.

2.3. Experimental procedure

The incubation procedure and measurements of esterification of [1-^{14}C]oleate and its conversion to $^{14}CO_2$ were as in [17]. Acetoacetate, hydroxybutyrate, glucose, pyruvate and lactate were determined in neutralized HClO$_4$ extracts by enzymic methods [18–20]. The metabolite changes and $^{14}CO_2$ production over 20–60 min were calculated from plots of the values at 20, 40 and 60 min. cAMP was determined in KOH-neutralized HClO$_4$-treated hepatocyte extracts as in [21], using a kit from the Radiochemical Centre. The HClO$_4$ extracts were made 4 min after addition of the hormones.

Statistical significance of results was assessed using Student's unpaired *t*-test except where indicated.

Table 1

Comparison of the effects of adrenaline and noradrenaline on oxidation of $[1\text{-}^{14}C]$oleate to $^{14}CO_2$ and cAMP levels in hepatocytes from fed rats in the absence or presence of $CaCl_2$

Addition	$-CaCl_2$		$+CaCl_2$	
	$[1\text{-}^{14}C]$Oleate oxidized to $^{14}CO_2$	cAMP content (pmol/g fresh wt cells)	$[1\text{-}^{14}C]$Oleate oxidized to $^{14}CO_2$	cAMP content (pmol/g fresh wt cells)
None	29.5 ± 1.1 (8)	5.88 ± 0.30 (4)	29.5 ± 2.4 (8)	5.51 ± 0.33 (6)
Adrenaline (1 μg/ml)	31.9 ± 1.1 (6)	10.40 ± 1.78 (4)[a]	42.0 ± 1.6 (6)[c]	8.81 ± 0.52 (4)[d]
Noradrenaline (1 μg/ml)	32.3 ± 1.7 (6)	9.77 ± 1.33 (4)[a]	41.1 ± 3.8 (6)[b]	7.55 ± 0.37 (6)[c]

[a] $p < 0.05$ (paired t-test); [b] $p < 0.05$; [c] $p < 0.01$; [d] $p < 0.001$

Production of $^{14}CO_2$ is expressed as nmol . min^{-1} . g fresh wt $cells^{-1}$ and the results are mean values ± SEM with no. obs. in parentheses. Oleate was 1 mM and $CaCl_2$ 2.4 mM when present

3. Results

3.1. Effects of adrenaline and noradrenaline on oxidation of $[1\text{-}^{14}C]$oleate to $^{14}CO_2$

The addition of adrenaline (1 μg/ml) and noradrenaline (1 μg/ml) to hepatocytes from fed rats in the presence of Ca^{2+} (2.4 mM) increased oxidation of $[1\text{-}^{14}C]$oleate to $^{14}CO_2$ by 42.4% and 39.3%, respectively (table 1). The increases were not observed in the absence of added Ca^{2+}. In contrast, the catecholamines increased hepatocyte cAMP content in both the presence and absence of Ca^{2+} (table 1). Therefore the increases in $[1\text{-}^{14}C]$oleate oxidation to $^{14}CO_2$ are not mediated by an increase in cAMP content. Indeed dibutyryl-cAMP decreases oxidation of $[1\text{-}^{14}C]$oleate to $^{14}CO_2$ in hepatocytes from fed rats [14].

The effects of adrenaline and noradrenaline on $[1\text{-}^{14}C]$oleate oxidation to $^{14}CO_2$ were mimicked by phenylephrine (100 μg/ml) a synthetic catecholamine and α-agonist. Oxidation of $[1\text{-}^{14}C]$oleate to $^{14}CO_2$ was increased by phenylephrine in the presence of Ca^{2+} (control (5), 25.6 ± 2.4 nmol . min^{-1} . g fresh wt $cells^{-1}$; plus phenylephrine (5) 35.2 ± 3.2 nmol . min^{-1} . g fresh wt $cells^{-1}$, $p < 0.05$) but not in the absence of Ca^{2+} (control (5), 28.5 ± 1.9 nmol . min^{-1} . g fresh wt $cells^{-1}$; plus phenylephrine (5) 31.8 ± 1.9 nmol . min^{-1} . g fresh wt $cells^{-1}$).

Adrenaline and noradrenaline did not increase $^{14}CO_2$ production from $[1\text{-}^{14}C]$oleate in the presence of the α-blocking agent phentolamine (table 2). The basal (no hormone) rate of $^{14}CO_2$ production was unaffected by phentolamine (not shown). The β-antagonist propranolol did not diminish the effects of the catecholamines on $^{14}CO_2$ production (table 2) and

rates of $^{14}CO_2$ production were maximal in the presence of both propranolol and either adrenaline or noradrenaline (table 2). The increased $^{14}CO_2$ production observed on addition of propranolol in the presence of either catecholamine (table 2) may in part result from the propranolol blockade of cAMP accumulation in response to the catecholamines (not shown). Thus propranolol in the absence of Ca^{2+} in the presence of either catecholamine causes small (but not significant) increases in $^{14}CO_2$ production (adrenaline alone (4), 32.5 ± 0.7 nmol . min^{-1} . g fresh wt $^{-1}$; adrenaline + propranolol (4), 38.3 ± 3.8

Table 2

Effects of phentolamine and propranolol on oxidation of $[1\text{-}^{14}C]$oleate to $^{14}CO_2$ in hepatocytes from fed rats in the presence of adrenaline or noradrenaline

Addition	$[1\text{-}^{14}C]$Oleate oxidized to $^{14}CO_2$
None	30.9 ± 1.7 (4)
Adrenaline (1 μg/ml)	43.9 ± 1.0 (4)[d]
+ phentolamine (30 μg/ml)	31.2 ± 2.0 (4)
+ propranolol (30 μg/ml)	52.7 ± 3.2 (4)[d]
None	27.1 ± 3.5 (5)
Noradrenaline (1 μg/ml)	38.6 ± 3.4 (5)[a]
+ phentolamine (30 μg/ml)	30.6 ± 3.6 (5)
+ propranolol (30 μg/ml)	46.3 ± 4.9 (5)[b]

[a] $p < 0.05$ (paired t-test); [b] $p < 0.01$ (paired t-test); [c] $p < 0.05$; [d] $p < 0.001$; Values significantly different from those of the control

Production of $^{14}CO_2$ is expressed as nmol . min^{-1} . g fresh wt $cells^{-1}$ and the results are mean values ± SEM with no. obs. in parentheses. Oleate was 1 mM and $CaCl_2$ 2.4 mM

Table 3

Effects of adrenaline and noradrenaline on ketogenesis, glucose and lactate plus pyruvate production in hepatocytes from fed rats

Addition	$-CaCl_2$			$+CaCl_2$		
	Ketone bodies	Glucose	Lactate + pyruvate	Ketone bodies	Glucose	Lactate + pyruvate
None	0.45 ± 0.05 (8)	1.42 ± 0.09 (8)	0.31 ± 0.04 (6)	0.49 ± 0.06 (8)	1.48 ± 0.07 (8)	0.25 ± 0.03 (6)
Adrenaline (1 µg/ml)	0.42 ± 0.06 (6)	2.24 ± 0.05 (6)[c]	0.20 ± 0.03 (6)[a]	0.45 ± 0.07 (6)	2.54 ± 0.14 (6)[c]	0.20 ± 0.03 (6)[a]
Noradrenaline (1 µg/ml)	0.51 ± 0.06 (6)	2.18 ± 0.13 (6)[c]	0.19 ± 0.03 (6)[a]	0.50 ± 0.06 (6)	2.56 ± 0.20 (6)[b]	0.21 ± 0.04 (6)

[a] $p < 0.01$ (paired); [b] $p < 0.01$; [c] $p < 0.001$; values significantly different from those without addition of hormone

For details see section 2. Oleate (1 mM) was present in all incubations. Production of ketone bodies, glucose and lactate plus pyruvate is expressed as μmol . min^{-1} . g fresh wt cells^{-1}

nmol . min^{-1} . g fresh wt^{-1}; noradrenaline alone (5), 30.8 ± 1.1 nmol . min^{-1} . g fresh wt^{-1}; noradrenaline + propranolol (5) 34.7 ± 2.9 nmol . min^{-1} . g fresh wt^{-1}). Addition of propranolol in the absence of hormone also caused variable increases (up to 33%) in $^{14}CO_2$ production. The reason for this increased $^{14}CO_2$ production is not known.

3.2. *Effects on ketogenesis and glucose production*

Addition of adrenaline or noradrenaline increased glucose release to the medium (table 3); this was presumably due to activation of glycogenolysis. The increased glucose release was observed both in the presence and absence of added Ca^{2+}, which suggests that in the absence of Ca^{2+} glycogenolysis was mediated by the increased cAMP concentrations (see table 1). In support of this, catecholamine-stimulated glucose release in the absence of Ca^{2+} was diminished on addition of propranolol (not shown). Production of lactate and pyruvate was slightly inhibited by adrenaline and noradrenaline (table 3). The catecholamines were without effect on the rate of ketogenesis from oleate in either the presence or absence of Ca^{2+} (table 3). Catecholamine-induced increases in glucose release (table 3) and $^{14}CO_2$ production from [1-^{14}C]-oleate were observed in the same experiments showing that the cells were responsive to the hormones.

4. Discussion

We have shown that vasopressin stimulates $^{14}CO_2$ production from [1-^{14}C]oleate in a Ca^{2+}-dependent manner [14]. These results demonstrate that adrenaline and noradrenaline similarly stimulate $^{14}CO_2$ production from [1-^{14}C]oleate by an α-adrenergic, cAMP-independent mechanism.

As [1-^{14}C]oleate is metabolized to [1-^{14}C]acetyl CoA increased production of $^{14}CO_2$ from [1-^{14}C]-oleate could result from increased tricarboxylic acid flux. Supporting this, stimulation of α-receptors markedly increases O_2-consumption by the perfused rat liver [22]. The Ca^{2+} requirement for α-adrenergic stimulation of $^{14}CO_2$ production from [1-^{14}C]oleate implies that a change in mitochondrial Ca^{2+} uptake may be involved in the mechanism. Perfusion of rat liver with α-adrenergic agonists does induce a stable modification of the mitochondria which leads to increased Ca^{2+} uptake and retention [23]. As enzymes of the tricarboxylic acid cycle, NAD isocitrate

dehydrogenase (EC 1.1.1.4.1) and the 2-oxoglutarate dehydrogenase complex, are activated by low [Ca^{2+}] [24,25] it is tempting to speculate that the catecholamines exert their effects on [1-^{14}C]oleate oxidation to $^{14}CO_2$ by activating these enzymes via an increase in mitochondrial Ca^{2+}. Alternatively the increased $^{14}CO_2$ production may be secondary to stimulated respiratory chain activity. Mitochondria prepared from hepatocytes (from starved rats) treated with catecholamines show increased rates of ADP-dependent respiration and mitochondrial ATPase activity, and these effects are α-mediated [26–28].

The lack of effect of the catecholamines on ketogenesis from oleate (table 3) is in marked contrast to the decreased ketogenesis from oleate observed in the presence of vasopressin [14,29]. In this case the increased oxidation of oleate to CO_2 contributes to the antiketogenic effect of the hormone [14]. However, it should be noted that part of the antiketogenic action of vasopressin may be related to increased glycolytic flux [14,29] whereas adrenaline and noradrenaline tend to decrease output of lactate and pyruvate (table 3). The interpretation of the results obtained with the catecholamines is made difficult by the increases in cAMP content that occur concomitantly with the α-response (table 1). These increases in cAMP content might be expected to increase ketogenesis as glucagon and dibutyryl cAMP increase ketogenesis [29–31]. However the increases in cAMP elicited by the catecholamines are much smaller (5–10-times smaller) than those elicited by glucagon (unpublished, [32]). Small stimulatory effects of adrenaline on ketone body production by perfused livers of fed rats have been observed in [33,34].

It is not known to what extent the effects of the catecholamines on $^{14}CO_2$ production from [1-^{14}C]-oleate may be secondary to changes in the rate of removal of [1-^{14}C]oleate from the medium or the rate of entry of oleoyl-CoA into the mitochondria. It is unlikely that the increased $^{14}CO_2$ production is secondary to increased extraction of free fatty acid since both adrenaline and noradrenaline decreased the extraction of free fatty acids by the perfused rat liver [33]. However it is possible that the catecholamines may change the rate of entry of long-chain fatty acyl CoA into the mitochondria. Adrenaline inhibits acetyl-CoA carboxylase (EC 6.4.12) in the perfused rat liver [35] and we have found that noradrenaline (1 μg/ml) also inhibits acetyl-CoA carboxylase activity (assayed both with and without

preincubation with potassium citrate to activate the enzyme) in extracts of rat hepatocytes (unpublished). Such inhibition of acetyl-CoA carboxylase could decrease the concentration of malonyl-CoA, an inhibitor of entry of long-chain fatty acyl-CoA into the mitochondria [36,37]. However, we found no effect of adrenaline or noradrenaline on ketogenesis from oleate either in the presence or absence of Ca^{2+} (table 3) which might imply that if the rate of keto-genesis is governed solely by the mitochondrial uptake of free fatty acid [36,37] the Ca^{2+}-dependent activation by the catecholamines of $^{14}CO_2$ production from $[1-^{14}C]$oleate is not secondary to an increase in the mitochondrial uptake of fatty acyl CoA by this mechanism.

Acknowledgements

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